THE DERMATOPHYTE, *MICROSPORUM GYPSEUM*, AS A SAPROPHYTE AND PARASITE*

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Vanbreuseghem (1) recently described a selective procedure for isolating keratinophilic fungi from soil. The method extends the work of Karling (2), who first used keratin in various forms as bait for the isolation of chytrids from soil and water. Vanbreuseghem placed hair filaments upon the surface of moistened soil in which *Trichophyton mentagrophytes* (*Ctenomyces interdigitalis*), *T. rubrum* and *Epidermophyton floccosum* had been grown. After a few days, the hair bait became visibly overgrown by mycelium which was noted to be penetrating the hair shafts by means of "perforating organs" (Vanbreuseghem, 3).† Cultures made from the invaded hair yielded *T. rubrum* and *E. floccosum*, but were negative for *T. mentagrophytes*.

Previous attempts to isolate these dermatophytes directly from the test soils had ended in failure, due to overgrowth of the culture tubes by saprophytic molds, which had contaminated the soils during some prior manipulations. Vanbreuseghem examined a number of natural soils with his technic and isolated a keratinophilic fungus which subsequently was designated to be a member of a new genus *Keratinomyces* and given the specific name of *K. ajelloi* (4). However, none of the known dermatophytes was isolated from the Belgian soils studied.

The present paper records the successful isolation of a known dermatophyte from natural soils baited with hair.

MATERIALS AND METHODS

The soils examined in this study were collected from a wide variety of sites in Williamson County, Tennessee, and the counties of Cobb, DeKalb, Gilmer, Rabun and Thomas, Georgia. The Tennessee soils had been used previously in the course of a study of the occurrence and distribution of *Histoplasma capsulatum* in an endemic area of benign histoplasmosis (5–6). All the samples were gathered from the upper surface of the collection site by scooping them directly into 4 oz. bottles.

In the laboratory, sterile Petri dishes were half-filled with the soil specimens, moistened with 15 to 30 ml. of sterile distilled water (the exact amount dependent upon the nature of the soil samples), and baited by placing short tufts of autoclaved human hair upon the surface of the soil. These preparations then were incubated at room temperature (20–25°C) in a dark cupboard and examined periodically for the development of mycelium on the hair filaments.

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† The radial penetration of hair filaments by the mycelium of dermatophytes was first described by Davidson and Gregory in 1934 (130). These observers clearly noted the formation of pits, extending deep into the hair, as a result of the enzymatic activity of the invading hyphae.

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Hairs overgrown with mycelium were examined microscopically and cultured on a medium containing cycloheximide* ("acti-dione"), penicillin and streptomycin.

The isolation medium was prepared by adding 0.5 mg. of cycloheximide, 20 units of penicillin and 40 units of streptomycin per ml. of Sabouraud dextrose agar previously sterilized and cooled to 45°C. This medium had been primarily developed for the isolation of *Coccidioides immitis* from soil (7), but was subsequently found to permit the selective isolation of most of the fungi parasitic to man from a variety of heavily contaminated sources (Georg, 8; Ajello and Getz, 9).

FIG. 1. Soil plate showing appearance of *Microsporum gypseum* on hair filaments used as bait. (Soil sample 91A).

RESULTS

Of 74 Tennessee samples examined, 26, or 35.1%, yielded cultures of the dermatophyte *Microsporum gypseum* as did 11 of 42 Georgia specimens (26.2%), giving an over-all recovery of 31.9%.

In all instances, the growth of this dermatophyte upon the bait was vigorous and easily detected with the naked eye (Fig. 1). The hair filaments became covered with a yellowish-white mantle of mycelium. As seen in Fig. 2, the elliptical, echinulate, multiseptate macroconidia, diagnostic of *M. gypseum* were produced in great numbers. In addition, spherical, unicellular microconidia were also produced. The hair filaments were penetrated to varying depths by "perforating organs" (Fig. 3) which were composed of a cone-shaped mass of

* Generously donated by the Upjohn Co., Kalamazoo, Michigan.

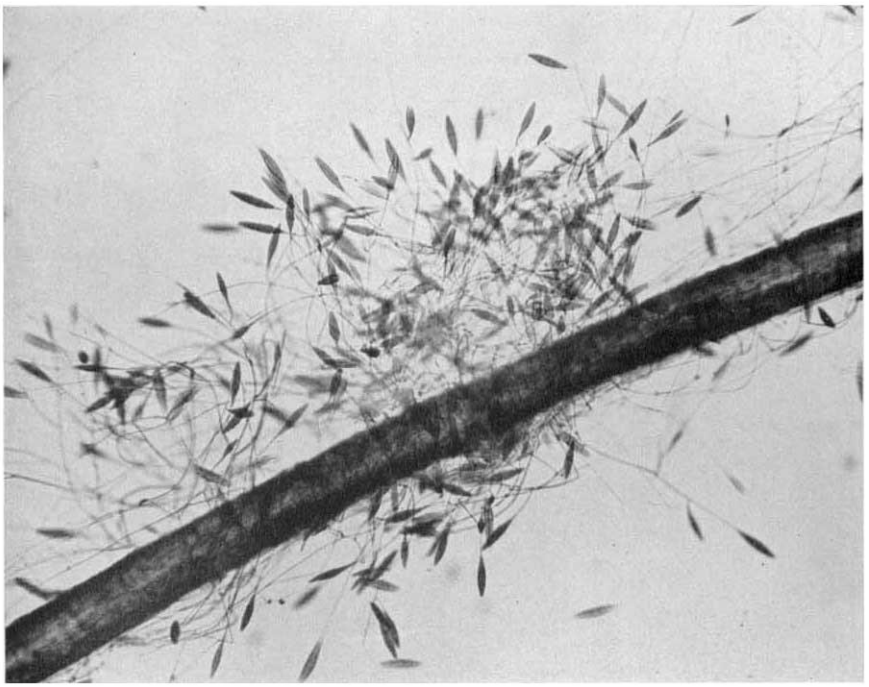

FIG. 2. Profuse production of macroconidia by *M. gypseum* on hair filaments exposed to soil (soil isolate G8). Original magnification 100×.

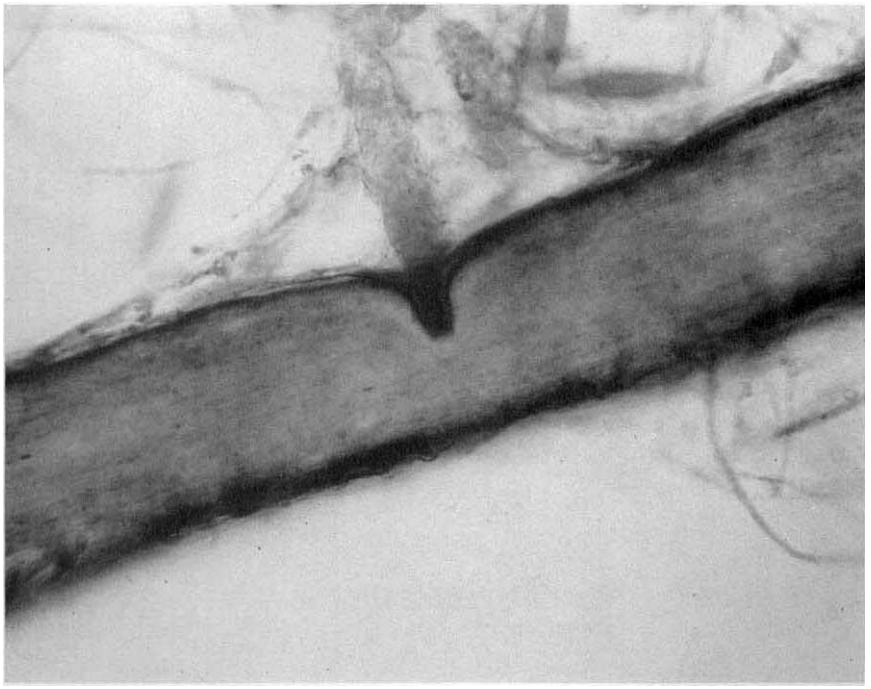

FIG. 3. Perforation of hair filament by mycelium of *M. gypseum*. (Isolate G8). Original magnification 475×.

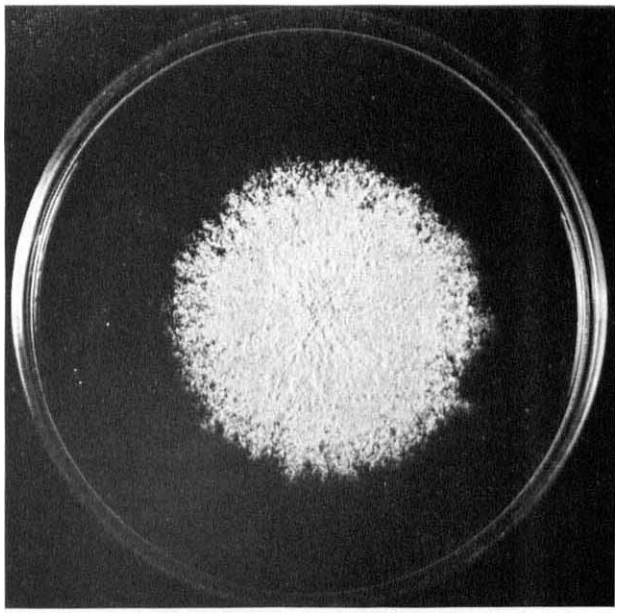

FIG. 4. Cultural appearance of *M. gypseum* on Sabouraud dextrose agar (Soil isolate G8)

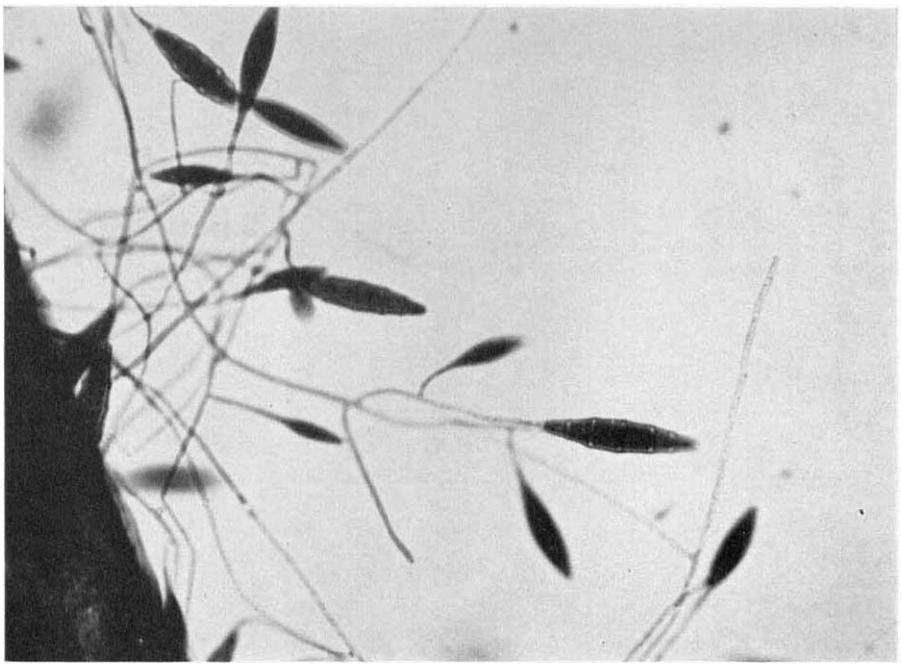

FIG. 5. Macroconidia of *M. gypseum* as produced on hair (Soil isolate G8). Original magnification 450×.

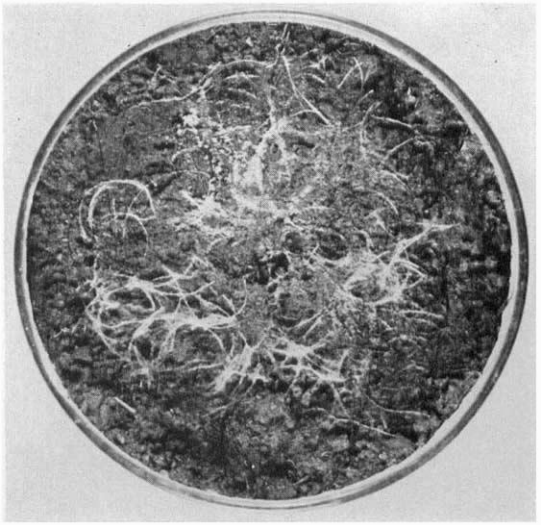

FIG. 6. Appearance of *Keratinomyces ajelloi* on hair filaments used as bait. (Soil sample G18).

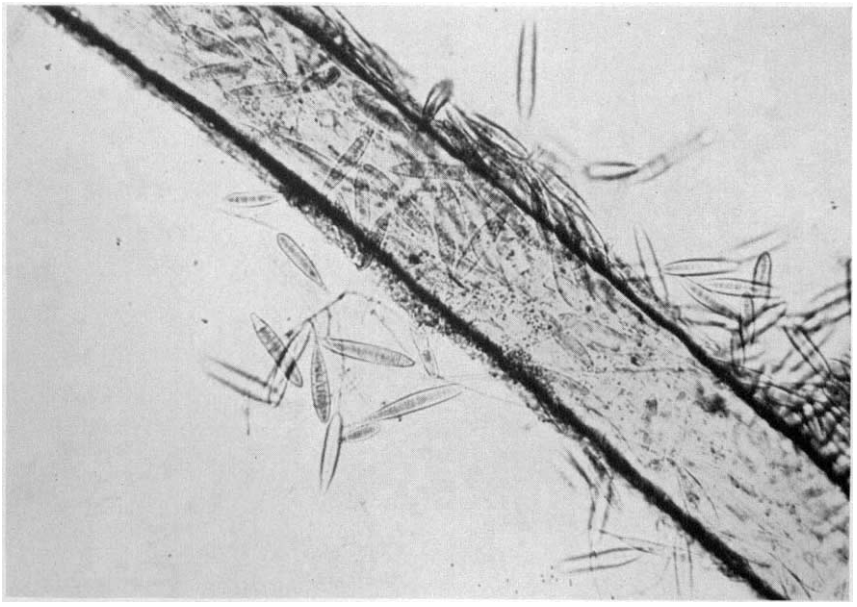

FIG. 7. Profuse production of macroconidia by *K. ajelloi* on hair filament. (Soil isolate G39). Original magnification 225×.

mycelium that grew in size as it progressively penetrated the hair filament, eventually perforating the shaft.

Through use of the selective isolation medium, pure cultures of the 37 isolates of *M. gypseum* were obtained. All produced powdery, cinnamon-brown colored

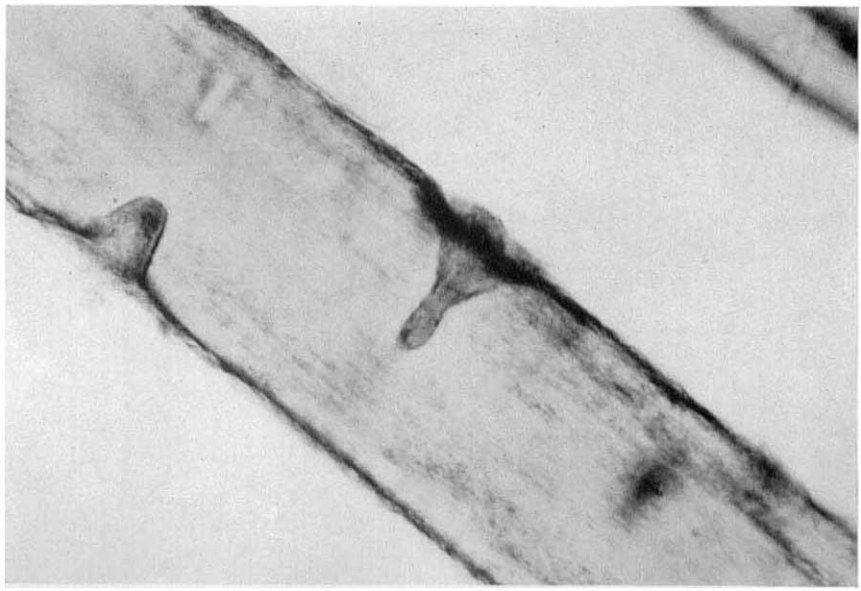

FIG. 8. Penetration of hair filament by *K. ajelloi* (Soil isolate G18). Original magnification 525×.

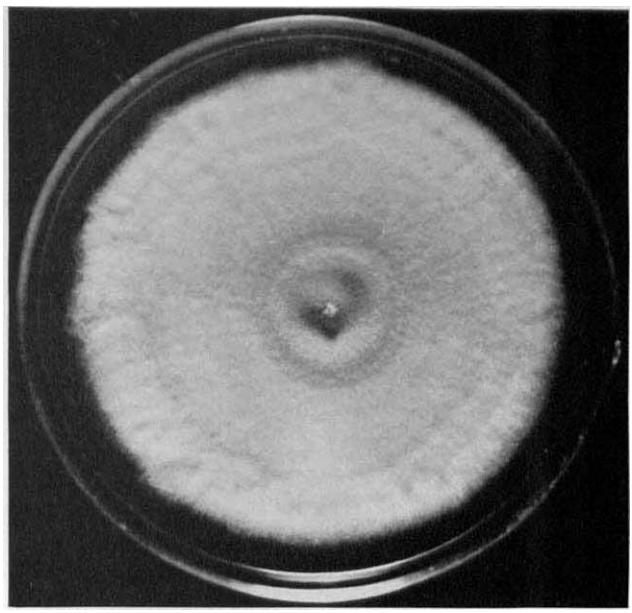

FIG. 9. Colony of *K. ajelloi* on Sabouraud dextrose agar (Soil isolate 100D)

colonies (Fig. 4) and abundant elliptical thin-walled, multiseptate macroconidia that are so typical of this fungus (Fig. 5).

It was not deemed necessary to determine the pathogenicity of these soil isolates, as Gordon (10) had previously verified on a human subject the infective

nature of a culture of *M. gypseum* directly isolated from one of the Tennessee soil samples.

Microscopic examination of the bait soon after exposure to the moistened soil frequently revealed the presence of phycomycetes representative of the orders Chytridiales and Peronosporales. But these rapidly disappeared and never developed to the extent that their growth became visible to the naked eye. A few fungi other than *M. gypseum* developed frank mycelial growth upon this substratum. These were species of *Fusarium*, *Scopulariopsis*, *Penicillium*, *Sepedonium*, etc. which could be looked upon as adventitious organisms that merely grew over the hair, never producing perforating organs nor bringing about its disintegration.

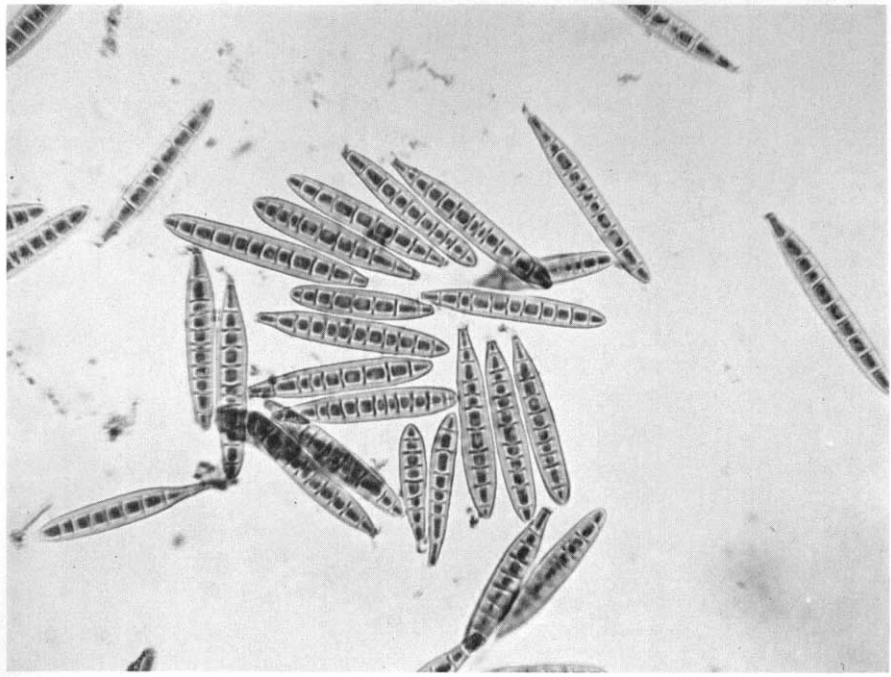

FIG. 10. Macroconidia of *K. ajelloi* (Soil isolate G18). Original magnification 450×

Others, representing several different fungi, not only produced abundant mycelium but also formed perforating organs. One of these, represented by 3 isolates, one from Tennessee and 2 from Georgia, was not a dermatophyte and proved to be Vanbreuseghem's *K. ajelloi* (Figs. 6–10). (Keratinolysis is thus not strictly a property of the dermatophytes; despite this, the baiting procedure is remarkably selective.) The New World isolates of *K. ajelloi* are morphologically similar to Vanbreuseghem's but differ in their failure to produce a diffusible pigment.

DISCUSSION

M. gypseum was first encountered by Sabouraud in 1894 (11) but at the time was simply referred to as the "Trichophyton du chien." It was first fully studied

and described by Bodin in 1907 (12), who, following a system of classification based principally on clinical criteria, placed it in the now obsolete genus *Achorion* as *A. gypseum*. In subsequent years, this parasite was reisolated by several investigators, who, failing to relate their isolates to Bodin's, gave it new names. The synonomy was lengthened still further by those who made untenable taxonomic improvisations so that today we have the lengthy synonomy presented in Table I. Not until 1928 was Bodin's fungus correctly placed in the genus *Microsporum* by Guiart and Grigorakis (13).

Dermatologists customarily have divided the members of the genus *Microsporum* (*M. audouini*, *M. canis*, *M. gypseum*) into two groups based upon their relative prevalence as parasites of humans and lower animals. Thus, *M canis*

TABLE I

Synonomy of Microsporum gypseum

Microsporum gypseum (Bodin) Guiart and Grigorakis 1928. (13)
Trichophyton du chien Sabouraud 1894. (11)
Achorion gypseum Bodin 1907. (12)
M. fulvum Sabouraud (Uriburu) 1909. (14, 15)
M. flavescens Horta 1912. (16)
A. serisei Cazalbou 1913. (17)
M. scorteum Priestly 1914. (18)
M. marginatum Cazalbou 1914. (19)
M. xanthodes Fischer 1918. (20)
Sabouraudites gypseus Ota and Langeron 1923. (21)
S. fulvus Ota and Langeron 1923. (21)
S. flavescens Ota and Langeron 1923. (21)
S. xanthodes Ota and Langeron 1923. (21)
Closterospora gypsea Grigorakis 1925. (22)
C. fulva Grigorakis 1925. (22)
Gymnoascus gypseus Nannizzi 1927. (23)
Microsporum sp. Nakamura 1931. (24)
Ectotrichophyton nakamurae Dodge 1935. (25)

and *M. gypseum* are referred to as "animal types" since they have been considered to be primarily parasites of lower animals, such as cats and dogs, which served as sources of human infections. In contrast, *M. audouini* is designated frequently as the "human or anthropophilic type" in the belief that it is predominantly a parasite of man* and infections are transmitted only by contact from person to person.

Of the three *Microsporum* species, *M. gypseum* has long been considered to be the rarest. Reports of *M. gypseum* infections indeed are few, particularly when contrasted with those of *M. audouini* and *M. canis*. A survey of all available records has revealed 155 instances of human infections attributable to *M. gypseum* in the United States and 115 in the remainder of the world (Table II).

* Intensive animal studies may prove otherwise as two instances of canine infections attributed to *M. audouini* are recorded in the literature (Sabouraud, 1908 (26) and Murrell, 1951 (27)).

It is obvious from these data that *M. gypseum* has a global distribution. No basis exists for considering that fungus to be native to Brazil and having been introduced from there into the United States and, inferentially, to other countries as stated by Lewis and Hopper (119).

It was surprising, in view of the alleged association of *M. gypseum* with lower animals, to find recorded only 61 instances of lower animal infections by this fungus (Table III).

The world-wide occurrence of human *M. gypseum* infections, the rarity of cases involving lower animals, and the discovery that this fungus is prevalent in

TABLE II

Geographic Distribution of human Microsporum gypseum *infections*
(*Literature references indicated in parentheses*)

NORTH, CENTRAL & SOUTH AMERICA		EUROPE	
Argentina	1 (14)	Austria	9 (90–92)
Brazil	9 (16, 28–30)	Belgium	1 (93)
Canada	8 (31–34)	Denmark	20 (94–96)
Panama	2 (35)	England	14 (97–98)
Puerto Rico	1 (36)	Finland	3 (99–100)
United States	155 (37–88)	France	6 (11, 12, 15, 101–103)
Uruguay	3 (89)	Germany	2 (20, 104)
		Hungary	4 (105–106)
Total no. of cases....	179	Italy	4 (107–110)
		Ireland	1 (111)
		Netherlands	1 (112)
		Spain	1 (113)
		Switzerland	3 (114–115)
		Total no. of cases...	69
AFRICA		ASIA	
Belgian Congo	1 (116)	Japan	1 (24)
AUSTRALIAN AREA			
New Zealand	19 (117–118)	Australia	1 (18)

World total, 270.

soil leads to the conclusion that soil must be considered the main source of human infection. Lower animals, thus, can no longer be implicated as the prime source of *M. gypseum*. They, like man, are infected from soil. Only infrequently are infections transmitted from animal to animal.

Indications that *M. gypseum* probably existed in soil as a saprophyte had been previously revealed by Mandels, *et al.* (125), who in carrying out laboratory studies on the deterioration of fabrics by molds, isolated this fungus from a piece of wool fabric buried in a soil sample of unknown origin. In 1952, Cooke (126) recovered this dermatophyte from wool buried in 3 soil study plots located

in Idaho and Washington. These observations, although suggestive, did not conclusively prove that *M. gypseum* existed as a saprophyte in soil. However, definitive proof of saprophytism was finally obtained by Gordon, *et al.* (127) when the characteristic macroconidia of *M. gypseum*, which never are produced on tissues of living animals, were demonstrated in a soil sample collected in Tennessee.

TABLE III

Reported animal infections by M. gypseum

(Total 61)

Horse, 50	Dog,
Belgium, 40 (132)	France, (11)
France, 7 (120, 121, 131, 133)	
Madagascar, 2 (17, 133)	Cat, 4
German, 1 (122)	Austria, 1 (92)
	France, 1 (103)
	Hungary, 1 (106)
	United States, 1 (70)
Monkey, 4	Tiger, 1
South Pacific, 3 (123) Macacus cynomolgus	Indochina, 1 (133)
Germany, 1 (124) M. cynomolgus	Chicken, 1
	Italy, 1 (107)

TABLE IV

Correlation of soil habitat with recovery of M. gypseum

HABITAT	NO. OF SAMPLES COLLECTED	NO. POSITIVE FOR *M. gypseum*	PERCENTAGE POSITIVE
Inside barn	9	8	88.8
Barnyard	15	10	66.6
Inside chicken house	15	2	13.3
Chicken yard	7	0	0
Chicken manure	4	0	0
Under house	14	3	21.4
Near house	22	9	40.9
In open	13	5	38.5
Bank of stream	1	0	0
Forest	13	0	0
Other	3	0	0
Total	116	37	31.9%

An analysis of the sites, from which the soil samples in this study were gathered reveals a significant correlation between the presence of *M. gypseum* in the sample and the collection of the soil from areas frequented by animals (Table IV). Most of the positive collection sites either were obviously populated by animals or were areas frequented by domestic or wild animals and thus presumably seeded with keratinaceous debris.

The observed facts lead one to conclude that *M. gypseum* is essentially a

soil-inhabiting organism. It must be considered as being one of the components of the complex mycoflora of the soil, which in contradistinction to most terricolous molds has the added ability, under appropriate conditions, to invade the keratinaceous tissues of living animals and produce disease. In nature, *M. gypseum* probably plays a highly specialized role, bringing about, along with other keratinophilic organisms, the microbiological breakdown of keratin into simple elements.*

These findings bring to mind the prescient words of Davidson and Gregory (129) who in 1933 stated: "it may well be that cast-off hairs and epidermal scales are the most important natural substrata on which ringworm fungi pass their saprophytic existence and produce spores capable of infecting new human and animal hosts."

SUMMARY

The dermatophyte *Microsporum gypseum* was isolated from 37 of 116 soil samples collected in Tennessee and Georgia.

There was a significant correlation between the positive samples and the presence of animals at the collecting site.

It is suggested that *M. gypseum* is essentially a soil inhabiting fungus that only rarely parasitizes animals.

In nature, *M. gypseum* may be considered to participate in the microbiological breakdown of keratinaceous debris.

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* The keratinolytic power of *M. gypseum* has been demonstrated *in vitro* by Mandels *et al.* (125) and Page (128).

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